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Soon after the discovery of diphtheria antitoxin, several investigators attempted to convert diphtheria toxin into antitoxin by the electrical current, and some went so far as to suppose that this method would supersede the costly and time-consuming process of immunizing animals. Smirnow<sup>1</sup> inoculated rabbits with half a cubic centimeter of a two to three days old broth culture of diphtheria bacilli, and twenty-four hours later, when the animals were sick, injected 10 cubic centimeters of the anodal fluid, obtained by passing a current for eighteen hours through diphtheria toxin. According to him, the animals were saved by the injections. Bolton and Pease<sup>2</sup> stated that two cubic centimeters of the anodal fluid obtained from diphtheria toxin neutralized ten minimal lethal doses of the toxin. It is a well-established fact that acids destroy diphtheria toxin more readily than alkalies, and hence it is to be considered that it was the acid at anode which in Bolton and Pease's experiments neutralized the toxin. The latter investigators believed that the electric current caused a rearrangement of the constituent atoms of the toxin molecule, so that antitoxin resulted; but they did not determine whether the toxin molecule moved with or against the current by virtue of the charge which it carried.

The first to undertake the determination of the electro-positive or electro-negative nature of diphtheria toxin and antitoxin was Römer.<sup>3</sup> Römer used a U-shaped tube and allowed the electrodes to dip into the toxin and antitoxin to be investigated. After the current had been passed for a stated interval of time, the fluid was pipetted from both branches of the tube simultaneously and tested

<sup>1</sup> Smirnow, *Berl. klin. Woch.*, 1892, xxxii, 645.

<sup>2</sup> Bolton and Pease, *Jour. of Exper. Med.*, 1896, i, 537.

<sup>3</sup> Romer, *Berl. klin. Woch.*, 1904, xli, 209.

on guinea-pigs for toxic or antitoxic properties. The results were entirely negative, since he was unable to determine whether the toxin or antitoxin particles traveled toward the cathode or anode. This failure was due primarily, we believe, to the destruction of the toxin and antitoxin by the products of electrolysis, brought about by the strong current employed, for Römer states that he used oil to get rid of the bubbles at the electrodes. Furthermore, the method of simultaneous pipetting very likely caused some mixing of the different portions of the fluid.

To avoid these errors in technique and to eliminate the effects of electrolytic products, we first used a modification of Bilitzer's cells, as shown in Diagram 1. The cells and connecting tubes were of

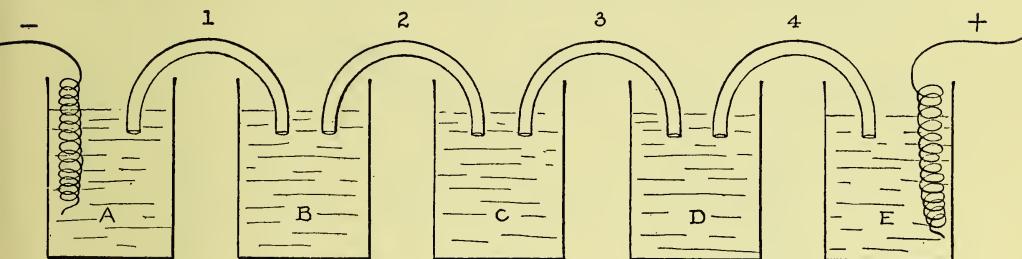


FIG. 1.

glass; the electrodes consisted of coils of platinum wire. The cells *A*, *B*, *D* and *E* were filled with distilled water, which was brought to the same level in all of them by means of water-filled connecting tubes. The central cell, *C*, was then filled to a slightly lower level than the others with the toxin or antitoxin to be tested. The small connecting tubes *1* and *4* being in place, and the direct lighting current turned on, the tubes *2* and *3* were filled with distilled water and simultaneously placed in position, thus completing the circuit. After the current had been passed for the desired length of time, the connecting tubes *2* and *3* were removed at the same moment, care being taken that they remained filled. Then *1* and *4* were removed. Under the influence of the current, the fluid in *A* became alkaline, that in *E*, acid, while *B* and *D* remained almost neutral. Hence, only the fluid in the two latter cells was tested on guinea-pigs for toxic or antitoxic value. The results

were unsatisfactory and contradictory, probably owing to the fact that some of the test fluid passed into the neighboring cells along the outside surface of the connecting tubes, being drawn up by capillarity. When we used longer connecting tubes, the internal resistance became too great for the passage of an appreciable amount of current. This method was therefore discarded.

The following apparatus yielded decisive results. Two semi-circular glass tubes, each 1 cm. in diameter and 20 cm. long, were filled with melted agar (2 per cent. agar in distilled water) and allowed to cool to the temperature of the room. These were then arranged as in Diagram 2, the same platinum electrodes being used as in the previous experiments. The toxin or antitoxin to be tested was placed in the middle beaker, distilled water, in the other two.

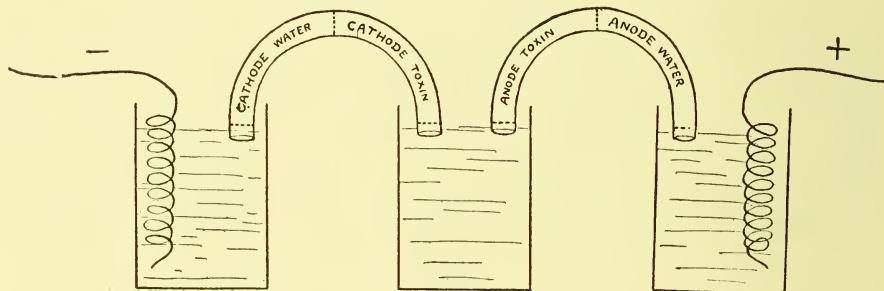


FIG. 2.

The direct Edison street lighting current, 110 volts, was passed for from four to five hours,<sup>4</sup> the distilled water in the end beakers being siphoned off and renewed every half hour to eliminate the disturbing influence of the products of electrolysis. The agar tubes were now removed and the ends thoroughly rinsed in distilled water. The agar mass was then forced from that portion of the tube which dipped into the toxin or antitoxin out through the other end. About a quarter of an inch was removed from each end of the mass, and the remainder divided approximately in half. Each

<sup>4</sup> The current was passed for only four or five hours because the risk of a disturbance due to products of electrolysis increased with the time; moreover, Bredig, Hardy, Pauli, and others found that the passage of a current for twenty-four hours or more would often cause a reversal of the charge carried by particles which would then necessarily be driven back in the opposite direction, thus obscuring the nature of the charge which they originally carried.

of these portions was rinsed in distilled water, chopped into fine pieces, and allowed to stand for one hour in about 6 c.c. of distilled water. At the end of this time the agar was removed by filtering through gauze, and the filtrate was tested on guinea-pigs

TABLE I.

Substance Tested by Passage of Electric Current.	Acidity or Alkalinity.	Cathode Water.	Cathode Toxin or Antitoxin.	Anode Toxin or Antitoxin.	Anode Water.
Diphtheria toxin.	Acid. Alkaline.	No reaction. No reaction.	+ in 20 hours. + in 28 hours.	No reaction. No reaction.	No reaction. No reaction.
Tetanus toxin.	Acid. Alkaline.	No reaction. No reaction.	+ in 48 hours. + in 40 hours.	No reaction. No reaction.	No reaction. No reaction.
Diphtheria anti-toxic serum. Tested against 35 M. L. D.'s.	Acid. Alkaline.	Protected. Protected.	Protected. Protected.	+ in 22 hours. + in 42 hours.	+ in 24 hours. + in 38 hours.
Diphtheria anti-toxic globulins. <sup>5</sup> Tested against 35 M. L. D.'s.	Acid. Alkaline.	+ in 36 hours. + in 24 hours.	Protected. Protected.	+ in 36 hours. + in 36 hours.	+ in 36 hours. + in 36 hours.
Tetanus antitoxic serum. Tested against 35 M. L. D.'s.	Acid. Alkaline.	Protected. Protected.	Protected. Protected.	+ in 50 hours. + in 48 hours.	+ in 68 hours. + in 48 hours.
Normal broth. No toxin.	Acid. Alkaline.	No reaction. No reaction.	No reaction. No reaction.	No reaction. No reaction.	No reaction. No reaction.
Normal horse serum. No an- titoxin. Tested against 35 M. L. D.'s.	Acid. Alkaline.	+ in 36 hours. + in 40 hours.	+ in 36 hours. + in 42 hours.	+ in 36 hours. + in 40 hours.	+ in 36 hours. + in 40 hours.
Test without the electric current. Diphtheria toxin. Diphtheria antitoxin. <sup>5</sup> Tested against 35 M. L. D.'s.			Water half negative + in 40 hours.		Toxin half negative + in 41 hours.

The first .5 cm. of the agar dipping into the toxin or antitoxin was removed, as was always done in our experiments with the electric current. There was always a slight trace of toxin or antitoxin in the first half centimeter, but it was never found beyond this point. A similar phenomenon was observed by Flexner and Noguchi, in relation to the diffusion of tetanus toxin into agar, for toxic or antitoxic properties. The accompanying table gives the results of these experiments.

<sup>5</sup> Gibson, *Jour. of Biol. Chem.*, 1905, i, 161; Flexner and Noguchi, *Jour. of Exper. Med.*, 1906, viii, 547.

In order that the table may be more readily understood, a detailed explanation of one of the experiments (No. 6, for example) contained in it may not be superfluous.

Diphtheria antitoxic serum was made slightly alkaline to phenolphthalein by the addition of a small amount of 0.1 normal sodium hydrate. The agar tubes were placed in position, as in Diagram 2, and the current was passed through it for four hours. The four portions of agar, which we shall designate as cathode water, cathode-antitoxin, anode water, and anode-antitoxin, were then cut into fine pieces and extracted in water for one hour, and to each of these extracts was added 35 m. l. d. of diphtheria toxin. These four portions of fluid were now injected subcutaneously into the abdominal walls of four guinea-pigs each weighing about 250 grams.

The two guinea-pigs receiving the fluid from the anode water and anode-antitoxin portions of agar died in thirty-eight and forty-two hours, respectively; hence, these portions must have contained no antitoxin. Those receiving the fluid from the cathode water and cathode-antitoxin portions showed no induration at the point of injection and no loss of weight for five days, after which they were discharged. Hence, they were fully protected against the 35 m. l. d. of toxin.

The table shows that under the influence of an electric current, the particles of both toxin and antitoxin travel toward the cathode, and that a change in the reaction of the solvent does not cause a reversal of the charge carried by the particles.

Blitz, Much and Siebert<sup>6</sup> claim that both tetanus toxin and tetanus antitoxin are precipitated by electro-positive inorganic colloids and conclude, therefore, that they are electro-negative and should, under the influence of an electric current, pass toward the anode. However, neither they nor Römer were able to demonstrate this fact experimentally. It is probable that the precipitates which they obtained were due either to the action of electrolytes contained in the toxin or antitoxin on their inorganic colloids, or to the action of these colloids on non-toxic or non-antitoxic protein substances, or to a combination of both. It is worthy of note that their non-

<sup>6</sup> Blitz, Much and Siebert, *Beit. zu exper. Therapie*, 1905.

toxic broth gave precipitates with practically the same inorganic colloids as did the toxin.

Hardy<sup>7</sup> states that proteins are amphoteric; that is, that in an acid medium they travel toward the cathode and in an alkaline medium toward the anode, while in a neutral medium they do not move toward either pole. He worked with an albumen coagulated by heat. Pauli,<sup>8</sup> using a protein solution obtained by dialyzing serum from eight to ten weeks and filtering off the euglobulin, found, in agreement with Hardy, that the protein was electro-positive in an acid solution and electro-negative in an alkaline one.<sup>9</sup> We have shown that alteration of the reaction of the solvent does not change the character of the charge carried by particles of toxin or antitoxin. If proteins are amphoteric, as is generally believed, then this observation of ours would point to the non-protein nature of toxin and antitoxin. In one series of experiments, however, we have found that the portion of agar containing the toxin or antitoxin, that is, the cathode portion, was the only one to give the biuret reaction and this occurred when the test substance was in either an acid or an alkaline solution. If experiments which we have under way should verify the last results, it would indicate that native proteins may not be amphoteric and then the argument that we have advanced as to the non-protein nature of toxin and antitoxin would be invalidated.<sup>10</sup>

If the combination of toxin with antitoxin is a true chemical reaction, one would expect that under the influence of an electric current toxin would travel in one direction and antitoxin in the opposite direction. Such, however, was not found to be the case and we are, therefore, inclined to believe that this union is not a true chemical reaction, but a matter of adsorption, as was first suggested by Bordet, and has since been claimed by others.

<sup>7</sup> Hardy, *Jour. of Physiology*, 1899, xxiv, 288.

<sup>8</sup> Pauli, *Hofmeister's Beitr.*, 1906, vii, 531.

<sup>9</sup> Pauli denaturalized his proteids by the prolonged dialysis.

<sup>10</sup> Oppenheimer (Toxin and Antitoxin, 1903) in summing up the work on this subject concluded that toxin and antitoxins are of non-protein nature. Quite recently Osborn, Mendel and Harris (*Amer. Jour. Physiol.*, 1905, xiv, 259), working with ricin, have taken issue with him, having found that their purest product still gave protein reactions. As a matter of fact, nothing definite is known at present of the chemical nature of these various substances.

## CONCLUSIONS.

1. Both diphtheria and tetanus toxin and their antitoxins are electro-positive, that is, they pass to the cathode under the influence of an electric current.
2. The character of the charge is not altered by a change in the reaction of the solvent.
3. The combination of toxin and antitoxin would seem to represent not a true chemical reaction but the adsorption of one colloid by another.